B6(SJL)-*Apoe*$^{tm1.1(APOE*4)Adiuj}$/J

Stock No: 027894
Protocol 28933: Sanger sequencing Assay - Apoe<tm1.1(APOE*4)Msasn-E4/E3/E2
Version 1.2

Notes

E4= C/C
E2= T/T
E3= T/C
WT = no amplification

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Expected Results

Sequence

CCCGGCTGGGCGCGGACATGGAGGACGTG(c/t)GCGGCC
GCCTGGTGCAGTACCGCGG
CGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGC
TGCGGGTGCGCCTCGCCTCC
CACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGC
CGATGACCTGCAGAAG(c/t)GC
CTGGCAGTGTACCAGGCCGGGGCCC

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
24458		CAA ATC GGA ACT GGA GGA AC		Forward	A	
24459		CCC CGG CCT GGT ACA CTG		Reverse	A	

Reaction A

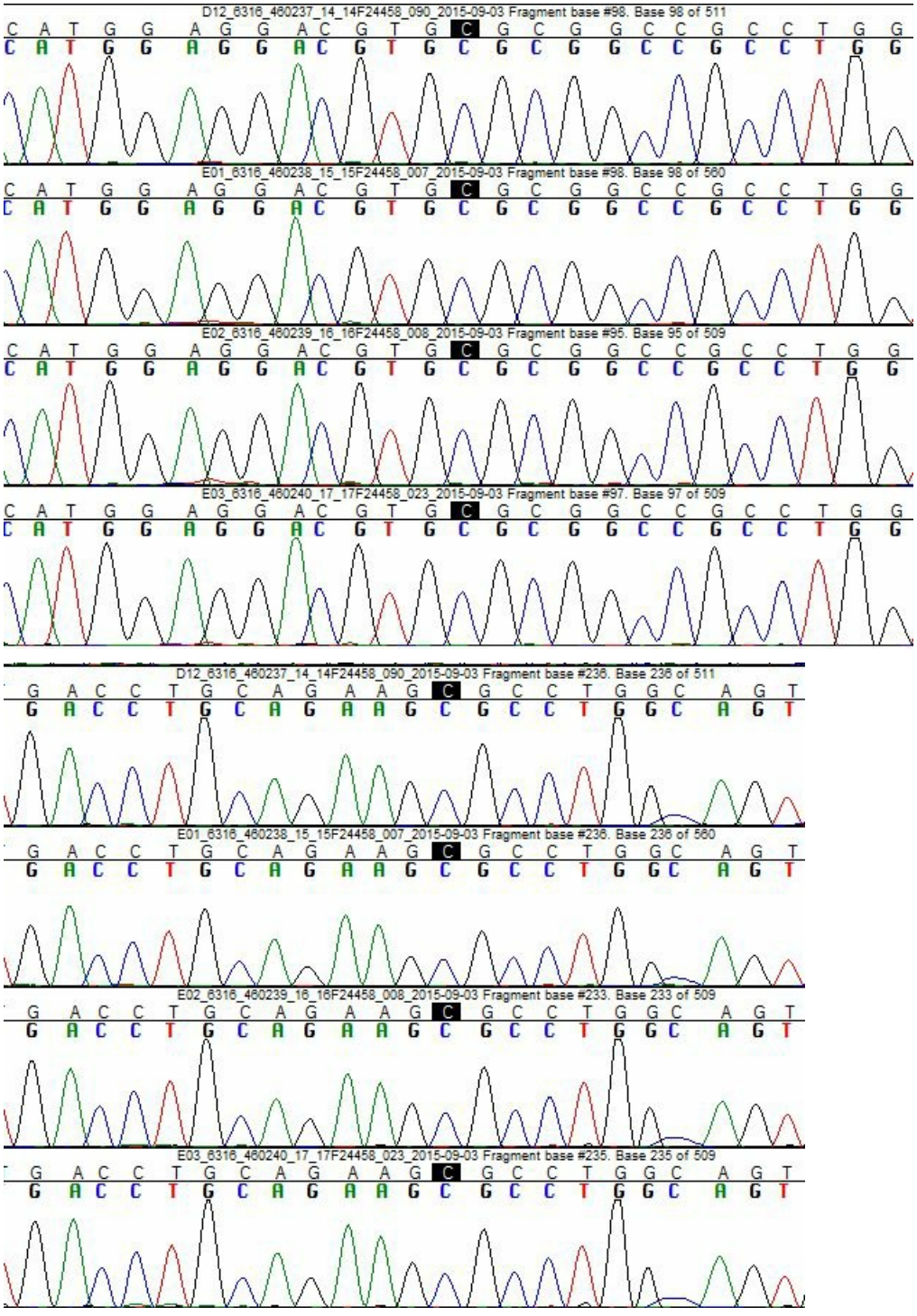
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTPS-kapa	0.26 mM
24458	0.50 uM
24459	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.


D12_6316_460237_14_14F24458_090_2015-09-03 Fragment base #98. Base 98 of 511
C A T G G A G G A C G T G C G C G G C C G C C T G G
E01_6316_460238_15_15F24458_007_2015-09-03 Fragment base #98. Base 98 of 560
C A T G G A G G A C G T G C G C G G C C G C C T G G
E02_6316_460239_16_16F24458_008_2015-09-03 Fragment base #95. Base 95 of 509
C A T G G A G G A C G T G C G C G G C C G C C T G G
E03_6316_460240_17_17F24458_023_2015-09-03 Fragment base #97. Base 97 of 509
C A T G G A G G A C G T G C G C G G C C G C C T G G
D12_6316_460237_14_14F24458_090_2015-09-03 Fragment base #236. Base 236 of 511
G A C C T G C A G A A G C G C C T G G C A G T
E01_6316_460238_15_15F24458_007_2015-09-03 Fragment base #236. Base 236 of 560
G A C C T G C A G A A G C G C C T G G C A G T
E02_6316_460239_16_16F24458_008_2015-09-03 Fragment base #233. Base 233 of 509
G A C C T G C A G A A G C G C C T G G C A G T
E03_6316_460240_17_17F24458_023_2015-09-03 Fragment base #235. Base 235 of 509
G A C C T G C A G A A G C G C C T G G C A G T